

PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Yeung et al.

Art Unit: Unassigned

Application No. Unassigned
(U.S. National Phase of PCT/US01/16187)

Examiner: Unassigned

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For: HIGH-THROUGHPUT METHODS OF
DISTINGUISHING AT LEAST ONE
MOLECULE INDIVIDUALLY IN A
SAMPLE COMPRISING MULTIPLE
MOLECULES AND SYSTEMS FOR USE
THEREIN

PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

1. A high-throughput method of distinguishing at least one molecule individually in a sample comprising multiple molecules, which method comprises:

- (i) subjecting a sample comprising multiple molecules, at least one molecule of which is detectably labeled, to electrophoresis,
- (ii) imaging the electrophoretic mobility of each detectably labeled molecule over time by detecting the position of the detectable label of each detectably labeled molecule over time and, optionally, at the same time, dispersing the imaging by a transmission grating for spectroscopic analysis, and
- (iii) determining the electrophoretic mobility of each detectably labeled molecule and, optionally, determining the molecular spectrum of each detectably labeled molecule,

thereby distinguishing at least one molecule individually in a sample comprising multiple molecules.

2. The high-throughput method of claim 1, wherein said multiple molecules in said sample are not amplified prior to being subjected to electrophoresis.

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3. The high-throughput method of claim 1, wherein said at least one molecule is a nucleic acid.
4. The high-throughput method of claim 3, wherein said nucleic acid is detectably labeled with a fluorescent label.
5. The high-throughput method of claim 1, wherein said at least one molecule is a protein.
6. The high-throughput method of claim 5, wherein said protein is detectably labeled with a fluorescent label.
7. The high-throughput method of claim 1, wherein said at least one molecule is a small molecule.
8. The high-throughput method of any of claim 1, wherein said sample comprises a buffer.
9. The high-throughput method of claim 8, wherein said buffer is photobleached.
10. The high-throughput method of claim 8, wherein said buffer comprises a sieving matrix.
11. The high-throughput method of claim 1, wherein said at least one molecule is detectably labeled with a fluorescent label and said fluorescent label is induced to fluoresce by a laser.
12. The high-throughput method of claim 11, wherein fluorescence from said fluorescent label is focused on an imaging means.
13. The high-throughput method of claim 12, wherein said imaging means is an intensified CCD camera.

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14. The high-throughput method of claim 12, wherein said laser generates extraneous light and said extraneous light is eliminated through the use of an equilateral prism and at least one optical pinhole positioned before said imaging means.

15. The high-throughput method of claim 12, wherein one or more optical filters are positioned in front of said imaging means.

16. The high-throughput method of claim 1, wherein said electrophoretic mobility is measured by a method selected from the group consisting of the multiframe method, the streak method and the multispot method.

17. The high-throughput method of claim 1, wherein said electrophoretic mobility is imaged in less than about 5 milliseconds.

18. The high-throughput method of claim 1, wherein the at least one detectably labeled molecule is present in said sample at a concentration of at least about 1 copy per milliliter.

19. The high-throughput method of claim 1, wherein at least about 200 detectably labeled molecules are imaged every 10 milliseconds.

20. The high-throughput method of claim 19, wherein at least about 2,500 detectably labeled molecules are imaged every 25 milliseconds.

21. A system for use in the method of claim 1, which system comprises:

(i) an electrophoretic sample channel, into which is introduced a sample comprising multiple molecules, at least one molecule of which is detectably labeled with a fluorescent label,

(ii) a light source comprising or consisting essentially of at least one wavelength of light that causes at least one molecule in said sample comprising multiple molecules that is detectably labeled with a fluorescent label to fluoresce, wherein said light source irradiates said electrophoretic sample channel,

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(iii) an imaging means, wherein said imaging means images the electrophoretic mobility of each detectably labeled molecule in said sample over time, and, optionally,

(iv) a transmission grating, which disperses the imaging of the electrophoretic mobility of each detectably labeled molecule in said sample.

22. The system of claim 21, which further comprises a lens between said light source and said electrophoretic sample channel, wherein said lens focuses said light at normal incidence to said electrophoretic sample channel.

23. The system of claim 21, wherein said laser generates extraneous light and said system further comprises an equilateral prism and at least one optical pinhole before said imaging means, wherein said equilateral prism and said at least one optical pinhole eliminate said extraneous light prior to it impinging on said imaging means.

57. The system of claim 22, wherein said laser generates extraneous light and said system further comprises an equilateral prism and at least one optical pinhole before said imaging means, wherein said equilateral prism and said at least one optical pinhole eliminate said extraneous light prior to it impinging on said imaging means.

58. The system of claim 21, wherein said imaging means is an intensified CCD camera.

59. The system of claim 58, which further comprises a microscope objective between said electrophoretic sample channel and said imaging means, wherein said microscope objective focuses the fluorescence from said fluorescent label onto said imaging means.

60. The system of claim 21, which further comprises one or more optical filters positioned in front of said imaging means.

61. The system of claim 22, which further comprises one or more optical filters positioned in front of said imaging means.

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62. The system of claim 21, wherein said imaging means images the electrophoretic mobility of a detectably labeled molecule in said sample in less than about 5 milliseconds.

63. The system of claim 21, wherein said imaging means images the electrophoretic mobility of at least about 200 detectably labeled molecules every 10 milliseconds.

64. The system of claim 21, wherein said imaging means images the electrophoretic mobility of at least about 2,500 detectably labeled molecules every 25 milliseconds.

65. A high-throughput method of distinguishing at least one molecule individually in a sample comprising multiple molecules, which method comprises:

- (vii) introducing a sample comprising multiple molecules in free solution, at least one molecule of which is detectably labeled, into a sample channel,
- (viii) simultaneously imaging the position of each detectably labeled molecule by detecting the position of the detectable label of each detectably labeled molecule and dispersing the imaging by a transmission grating for spectroscopic analysis, and
- (iii) determining the molecular spectrum of each detectably labeled

molecule,

thereby distinguishing at least one molecule individually in a sample comprising multiple molecules.

66. The high-throughput method of claim 65, wherein said multiple molecules in said sample are not amplified prior to being introduced into said sample channel.

67. The high-throughput method of claim 65, wherein said at least one molecule is a nucleic acid.

68. The high-throughput method of claim 67, wherein said nucleic acid is detectably labeled with a fluorescent label.

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69. The high-throughput method of claim 65, wherein said at least one molecule is a protein.

70. The high-throughput method of claim 69, wherein said protein is detectably labeled with a fluorescent label.

71. The high-throughput method of claim 65, wherein said at least one molecule is a small molecule.

72. The high-throughput method of claim 65, wherein said sample comprises a buffer.

73. The high-throughput method of claim 72, wherein said buffer is photobleached.

74. The high-throughput method of claim 65, wherein said at least one molecule is detectably labeled with a fluorescent label and said fluorescent label is induced to fluoresce by a laser.

75. The high-throughput method of claim 74, wherein fluorescence from said fluorescent label is focused on an imaging means.

76. The high-throughput method of claim 75, wherein said imaging means is an intensified CCD camera.

77. The high-throughput method of claim 75, wherein said laser generates extraneous light and said extraneous light is eliminated through the use of an equilateral prism and at least one optical pinhole positioned before said imaging means.

78. The high-throughput method of claim 75, wherein one or more optical filters are positioned in front of said imaging means.

79. The high-throughput method of claim 65, wherein said position is imaged in less than about 0.05 milliseconds.

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80. The high-throughput method of claim 65, wherein said at least one detectably labeled molecule is present in said sample at a concentration of at least about 1 copy per milliliter.

81. The high-throughput method of claim 65, wherein at least about 200 detectably labeled molecules are imaged every 0.10 milliseconds.

82. The high-throughput method of claim 81, wherein at least about 2,500 detectably labeled molecules are imaged every 0.25 milliseconds.

83. A system for use in the method of claim 65, which system comprises:

- (i) a sample channel, into which is introduced a sample comprising multiple molecules in free solution, at least one molecule of which is detectably labeled with a fluorescent label,
- (ii) a light source comprising or consisting essentially of at least one wavelength of light that causes at least one molecule in said sample comprising multiple molecules that is detectably labeled with a fluorescent label to fluoresce, wherein said light source irradiates said sample channel,
- (iii) an imaging means, wherein said imaging means images the position of each detectably labeled molecule in said sample, and,
- (iv) a transmission grating, which simultaneously disperses the imaging of the position of each detectably labeled molecule in said sample.

84. The system of claim 83, which further comprises a lens between said light source and said sample channel, wherein said lens focuses said light at normal incidence to said sample channel.

85. The system of claim 84, wherein said laser generates extraneous light and said system further comprises an equilateral prism and at least one optical pinhole before said imaging means, wherein said equilateral prism and said at least one optical pinhole eliminate said extraneous light prior to it impinging on said imaging means.

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86. The system of claim 83, wherein said imaging means is an intensified CCD camera.

87. The system of claim 86, which further comprises a microscope objective between said sample channel and said imaging means, wherein said microscope objective focuses the fluorescence from said fluorescent label onto said imaging means.

88. The system of claim 83, which further comprises one or more optical filters positioned in front of said imaging means.

89. The system of claim 83, wherein said imaging means images the position of a detectably labeled molecule in said sample in less than about 0.05 milliseconds.

90. The system of claim 83, wherein said imaging means images the position of at least about 200 detectably labeled molecules every 0.10 milliseconds.

91. The system of claim 83, wherein said imaging means images the position of at least about 2,500 detectably labeled molecules every 0.25 milliseconds.